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A NEW SPECIES OF EMERALD ASH BORER PARASITOID FROM CHINA BELONGING TO THE GENUS *TETRASTICHUS* HALIDAY (HYMENOPTERA: EULOPHIDAE)

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Abstract.—***Tetrastichus planipennisi* Yang, new species,** is described and illustrated based on individuals reared from mature larvae of the emerald ash borer, *Agrilus planipennis* Fairmaire (Buprestidae), collected from their galleries in the trunks of *Fraxinus mandshurica* Rupr. in northeastern China. *Tetrastichus planipennisi* is a gregarious endoparasite, with 56–92 individuals developing in a single host larva and observed parasitism rates from 32 to 65%. Based on reared field-collected material, the female to male ratio is 2.5:1. It is a koinobiont parasitoid and overwinters as a mature larva. The new parasitoid is a potential biocontrol candidate for *A. planipennis*.

Key Words: Hymenoptera, Eulophidae, *Tetrastichus planipennisi*, new species, emerald ash borer, *Agrilus planipennis* Fairmaire, parasitoid, biology, biocontrol

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is native to eastern Asia, including Japan, Korea, China, Inner Mongolia and the Russian Far East. EAB larval feeding damages ash trees (Oleaceae, *Fraxinus* spp.) by forming zigzag galleries in the phloem and outer sapwood. During heavy infestations, individual galleries coalesce, girdling and killing branches and/or entire trees. EAB mainly attacks *F. mandshurica* Rupr. and *F. rhychophylla* Hance. in northeastern China (Liaoning, Jilin, and Heilongjiang provinces) and *F. velutina* Torr. in northern China (Tianjin Municipality, Shandong, and Hebei provinces). Historically, EAB in China has been considered a minor pest, but our early investigations found in some locations, such as the seacoast forest-belt of Tianjin, nearly 80% of the ash trees were infested with mortality 35%. In 2002, EAB was discovered attacking ash trees in southeastern Michigan, and has since spread to adjacent areas of Windsor, Canada, northwestern Ohio (Lucas and DeFiance counties), and northeastern Indiana. In those areas, EAB is devastating both ornamental and forest trees (McCullough 2002, Plant Health Division 2005).

A joint cooperative program surveying for natural enemies of EAB in China was initiated in 2003 to screen potential biocontrol agents for use in North

America and China. Several parasitoids have been found, the first reported and laboratory maintained is *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang et al. 2005). The second candidate for an EAB biocontrol program is another species of parasitic wasp in the genus *Tetrastichus* Haliday (1844) (Hymenoptera: Eulophidae). This species is an abundant natural enemy of EAB in northeastern China with parasitism rates ranging from 32 to 65%. It is a koinobiont, allowing parasitized EAB larvae to continue development. Typical of other koinobionts, it is an endoparasite, and like many other eulophids, numerous larvae feed gregariously on a single host individual.

The Tetrastichinae can be distinguished from other Eulophidae by the following characters: postmarginal vein generally absent or much reduced; scutellum with two pairs of longitudinal grooves and 2 pairs of strong setae; female antenna with funicle 3-segmented, male antenna with funicle 4-segmented. *Tetrastichus* Haliday can be distinguished from other Tetrastichinae genera mainly by the combination of the following two characters: forewing with submarginal vein having usually 1 (rare 2) dorsal setae; propodeum with an inverted Y-shaped paraspiracular carina. Other characters to help define this genus are given by Graham (1991) and LaSalle (1994).

The genus *Tetrastichus* is cosmopolitan with 482 species (Noyes 2003). Graham (1991) revised the 45 European species and LaSalle (1994) listed 21 North American species. The literature on Asian species is less complete. Kostjukov (1995) produced a key for 155 species from the Russian Far East, describing many new species. In China 26 species have been reported and described by various authors (Liao et al. 1987; Sheng 1995; Sheng and Wang 1992, 1995; Sheng and Shen 1996; Yang 1996; Zhu and Huang 2001, 2002; He 2004). The following description of a new *Tetrastichus* species includes biological observations related to its EAB biocontrol potential.

Materials and Methods

During 2003 and 2004, surveys were conducted in Liaoning, Jilin, and Heilongjiang provinces for natural enemies of EAB. Stressed *F. mandshurica* with dead branches were located, most with trunk diameters of 5–15 cm. Portions of their trunk bark were peeled back to locate and trace the EAB larval galleries. When mature parasitoid larvae were located, they usually occupied the terminal end of their host gallery and laid adjacent to their mummified host. These wasp larvae and additional EAB larvae were collected and brought into the laboratory. Five wasp larvae and a piece of filter paper moistened with distilled water were placed into each vial (12 mm diameter × 74 mm length). Individual field sampled EAB larvae were placed in separate vials with a section of the ash twig (10 mm diameter and 60 mm length) with a trough carved between the bark and sapwood for the larva to feed and be observed. All vials were tightly plugged with cotton and maintained at ambient temperature (about 23°C) with the filter paper moistened every three days. During the EAB larval rearing, the bark flap over the twig trough was pulled back to observe the development of the EAB larva and determine if it was parasitized. When parasitoids emerged they were killed and point mounted for taxonomic study. Specimens were examined with an Olympus SZH10 Stereo Microscope. Micrographs were taken with a ZOEL 550LV scanning electron microscope. Morphological terms follow Bouček (1988) and Gibson (1997). Authorship of the new species is attributed solely to Yang Zhong-qi.

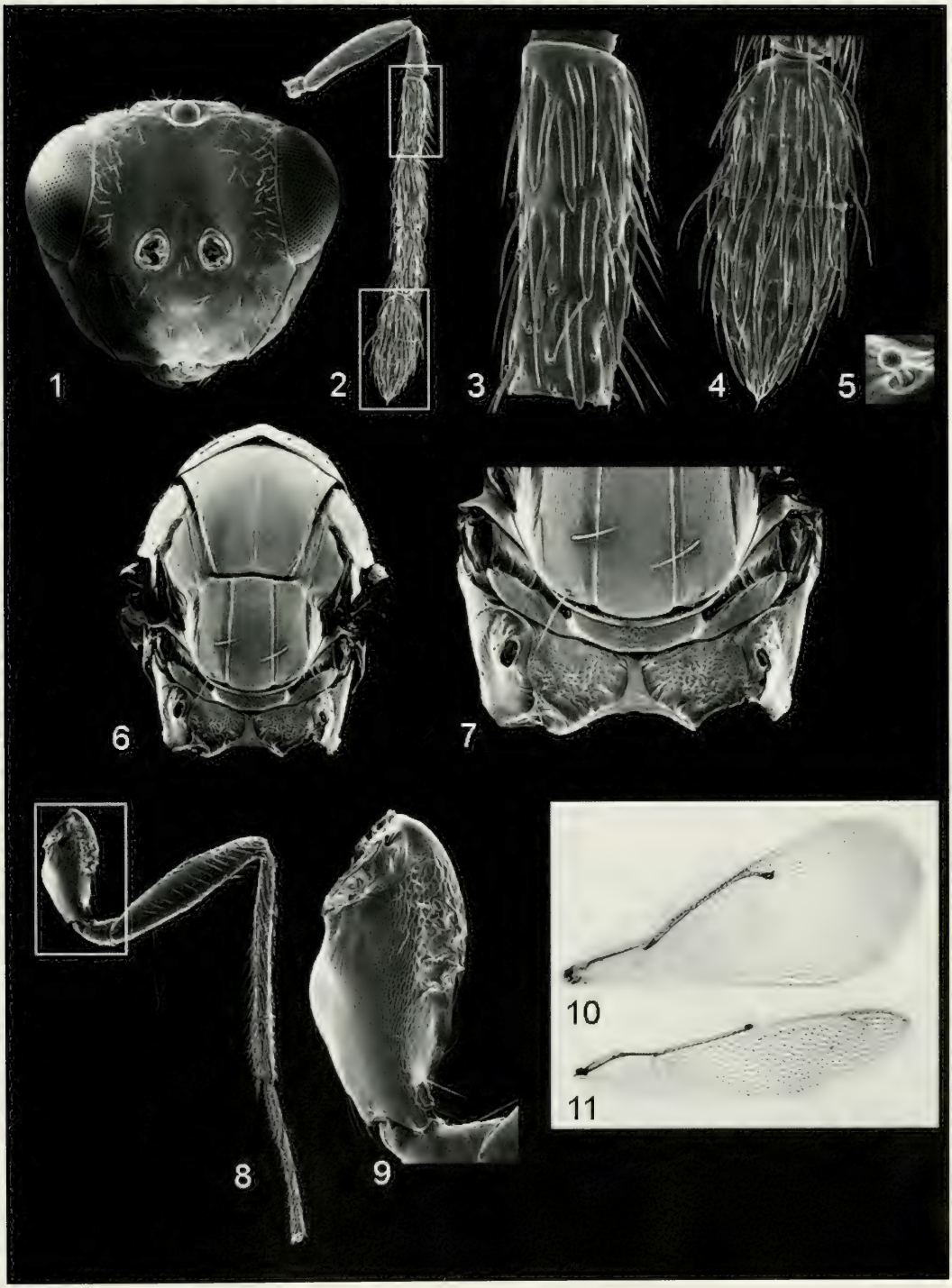
Tetrastichus planipennisi Yang, new species

(Figs. 1–26)

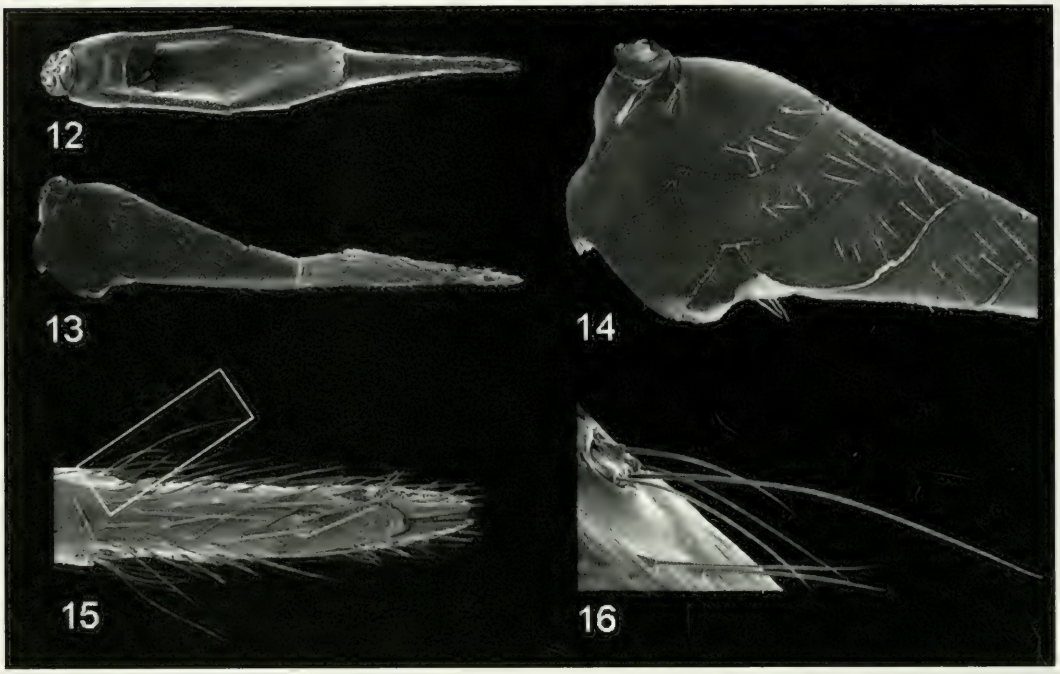
Female (Figs. 1–26).—Length 2.7–4.1 mm. Body dark green with bronze green tint. Antenna fuscous, except scape fulvous with apical 1/4 brownish and pedicel slightly reddish. Legs with coxae and femora same color as body except trochanters fuscous; tips of femora, tibiae and tarsi yellow, and last tarsal segment brownish. Wings hyaline, venation testaceous.

Head: Wide as mesosoma, in dorsal view width 2.3× length. POL 1.6 X OOL, OOL equals OD. Temple short, 0.22 length of eyes. Head (Fig. 1) anterior view slightly wider than tall (25:22). Scrobes moderately deep, nearly smooth. Parascrobal areas with numerous piliferous punctures near eyes. Eye sparsely pubescent, separated by 1.3–1.4× their length, length 1.9× width. Malar space 0.8 length of eye, malar sulcus nearly straight. Face medially convex. Mouth width 0.9–1.2× malar space. Clypeus with anterior margin deeply incised medially. Antennal (Fig. 1) toruli lower margin located on ventral line of eyes. Antenna (Fig. 2) with scape as long as eyes; scape length 4.8× its width, extending beyond median ocellus, reaching vertex; pedicel plus flagellum 1.2–1.28× width of head; pedicel 2.2× as long as wide, distinctly shorter than F_1 (5.5:9); funicle (Fig. 3) much thicker than pedicel; F_1 as broad as F_2 and F_3, but shorter than these segments (8:9:9); F_1 2.8 and F_{2-3} 3.0× as long as respective widths; clava (Fig. 4) slightly wider than flagellum (18:16), length 2.8× its width, distinctly shorter than F_2 plus F_3 (12.5:17), and with a weak constriction between C_1 and C_2; tip moderately acute; lengths of C_1 1.1 and C_2 0.8× their respective widths, C_3 trochiform as long as wide; terminal spine 0.3× length of C_3. Each funicle segment with 3 rows of longitudinal sensilla, and mushroom-like sensilla scattered on apical half (Fig. 5); clava with 2 rows of sensilla on C_{1-2}, and 1 on C_3, and the mushroom-like sensilla (Fig. 5) restricted to the apical margin of C_1.

Mesosoma: Moderately convex, length 1.4× width (Fig. 6). Midlobe of mesoscutum slightly shorter than wide (22:24), median line complete and posterior two-thirds distinct, 5–7 adnotaular setae present on each side. Mesoscutum and scutellum finely reticulate, areoles elongate Scutellum length 1.1–1.2× width, with submedian grooves separated from each other by 1.3× the distance either submedian groove from its nearest sublateral (= axillular) groove, area bordered by anterior and posterior margins of scutellum and submedian grooves with length 2.4× width; two pair of setae lateral to submedian grooves, first pair placed two-thirds along length of submedian groove from anterior margin, second pair near posterior margin, both pairs subequal in length to distance between submedian grooves. Propodeum (Fig. 7) length twice that of dorsellum, width 6.5× length, median area reticulate; median carina broad, adpetiolar strip well developed; spiracles ovate, distance from metanotum equal its length; callus with 6 setae anterior to spiracle and 2 posterior to spiracle. Prepectus moderately reticulate. Mesopleuron, mesepisternum and metapleuron reticulate. Legs slender; hind femur (Fig. 8) length 4.2× width, three rows of setae along length: dorsal and ventral margins, and anterior surface; hind coxa with posterior margin coarsely rugose (Fig. 9). Forewing (Fig. 10) costal cell 0.8× length of marginal vein and 9.4× width, bare above, with a row of hairs below; submarginal vein with 1–2 setae; marginal vein length 4.0× length of stigma, and 12.0× length of postmarginal vein; basal cell open, bare above, below with 2–4 hairs; basal setal line with



Figs. 1–11. *Tetrastichus planipennisi*, female. 1, Frontal view of head. 2, Antenna. 3, Funicle. 4, Clava. 5, Funicle sensilla. 6, Mesosoma, posterior half of scutellum and dorsellum. 7, Propodeum. 8, Hind leg. 9, Hind coxa. 10, Forewing. 11, Hindwing.

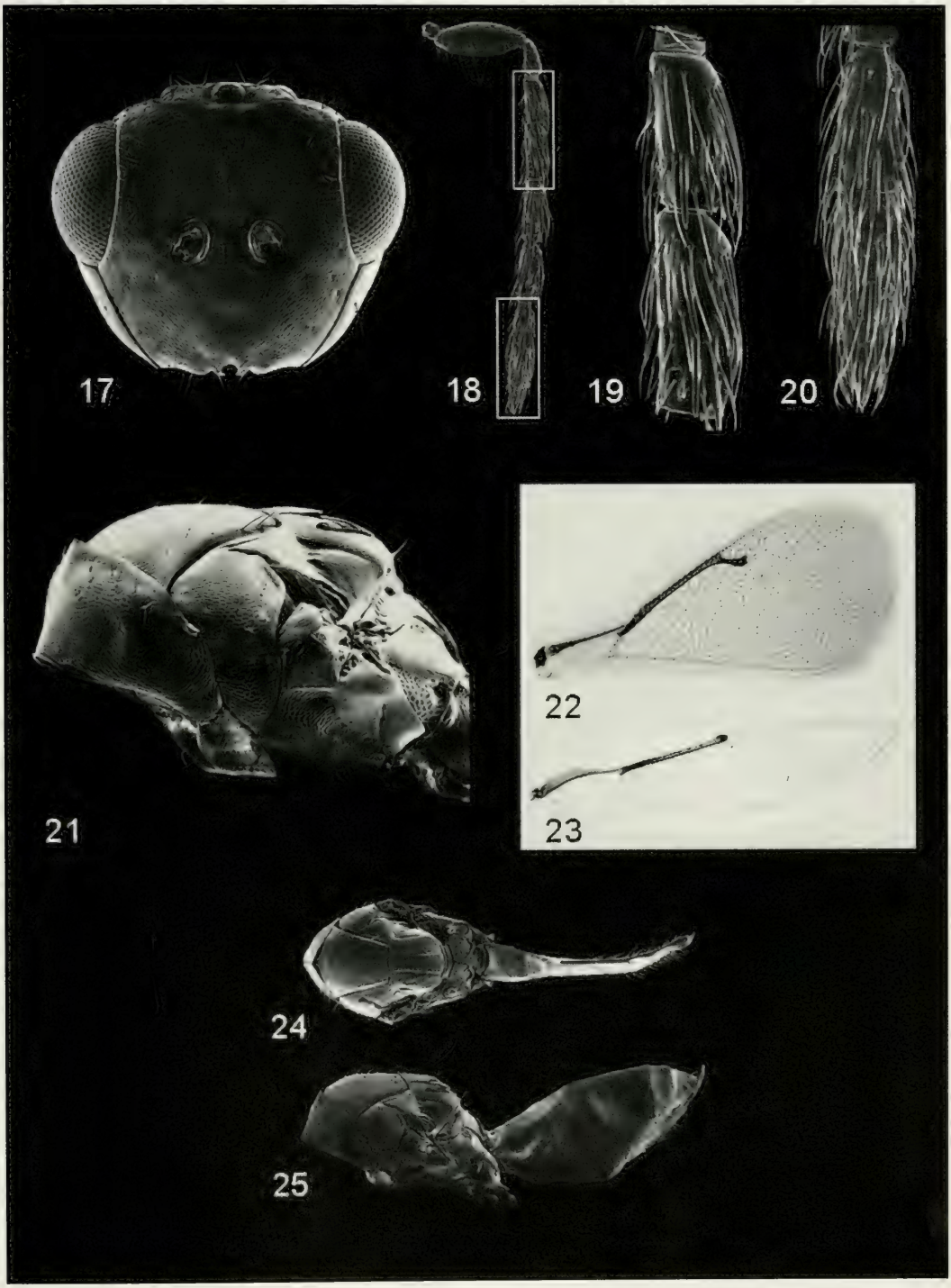


Figs. 12–16. *Tetrastichus planipennisi*, female. 12, Metasoma dorsal view. 13, Metasoma lateral view. 14, Metasoma lateral view of proximal half of metasoma. 15, Apex of last tergite. 16, Apex of last tergite four cercal setae.

about 9 setae; speculum small, extending as a narrow strip along two-thirds of marginal vein length, cubital setal line borders posterior margin. Hindwing as in Fig. 11.

Metasoma: Petiole ringlike, gaster (Figs. 12–13) in air dried specimens very long, strongly acuminate, depressed with dorsum sunken, 1.9–2.0× as long as head plus mesosoma, 5.0× as long as broad, and narrower than mesoscutum (27:32). All tergites delicately reticulate, and setae present except on dorsum of 1st and 2nd tergite. Tip of hypopygium (Fig. 14) at 0.12 length of gaster. Last tergite extremely long, 4.4–5.8× as long as basal broad; cercal setae (Figs. 15–16) with longest one about twice length of other three. Ovipositor projecting somewhat, 0.1 length of last tergite (Fig. 15).

Male (Figs. 17–25).—Differs from female as follows: Length 1.6–2.2 mm; body dark blue; scape black, wing venation fuscous. Frons with parascrobal areas having piliferous punctures much sparser (Fig. 17). Antenna (Figs. 18) with 4 funicular segments; scape moderately flattened, 2.5× as long as broad, and same length as eye, reaching vertex, with ventral plaque present medially and 0.64× as long as scape, with 5–7 long setae proximally along ventral edge; pedicel plus flagellum 1.6× width of head; pedicel 2.2× as long as broad, and shorter than F_1 (10:12); pedicel 0.80× length of F_1; F_1 0.76× as long as F_2; F_2–F_4 subequal in length, each about 2.5× as long as broad; each funicular segment slightly wider basally than apically and with a compact subbasal whorl of dark setae, those of F_1 extending beyond apex of segment (Fig. 19); clava (Fig. 20) acuminate, a little shorter than F_3 plus F_4 (28:30), 4.2× as long as broad, and as broad as F_4, C_1 1.2× length of C_2, C_2 slightly longer than C_3 (8:7); C3 shorter with terminal spine 0.3 length of segment; C_1 2.2 and C_2 1.6× as long as



Figs. 17–25. *Tetrastichus planipennisi*, male. 17, Frontal view of head. 18, Antenna. 19, Funicle 1 and 2. 20, Clava. 21, Mesosoma in lateral view. 22, Forewing. 23, Hindwing. 24, Metasoma in dorsal view. 25, Metasoma in lateral view.

Fig. 26. Mature larvae of *Tetrastichus planipennisi* visible through the integument of an *Agrilus planipennis* 4th instar larva.

broad. Mesosoma (Fig. 21) 1.7× as long as broad. Forewing (Fig. 22) with costal cell slightly shorter than marginal vein, latter 3.1× length of stigma; submarginal vein usually with 1 seta, occasionally 2. Gaster (Figs. 24, 25) in air dried specimens strongly compressed, slightly shorter than head plus mesosoma (66:70).

Etymology.—The specific epithet is derived from the specific name of its host, *Agrilus planipennis* Fairmaire.

Diagnosis.—The female is unique among other *Tetrastichus* having an extremely long gaster. Superficially similar to *Baryscapus agrilorum* (Ratzeburg), a parasitoid of Buprestidae (*Agrilus viridis* L.) in Germany (Graham 1991), the new species differs from *B. agrilorum* which has a curved malar sulcus; submarginal vein with 3–4 dorsal setae; midlobe of mesoscutum with 9–12 adnotaular setae, and last tergite of gaster shorter, 3.3–4.1× as long as wide. The new species is also close to *Tetrastichus telon* (Graham) (Graham 1961) from Europe, but the syntergum of this species is much shorter, 1.9–2.5× as long as broad and the submedian lines of scutellum are equidistant from each other and the lateral lines. Four other *Tetrastichus* species have been reported to parasitize *Agrilus* and other buprestid beetles including *T. ulmi* Erdös, 1954; *T. agrilocidus* Graham, 1991; *T. agrili* Crawford, 1914; and *T. jinzhouicus* Liao, 1987. *Tetrastichus planipennisi* females can be separated from these species by the long metasoma (1.9–2.0× as long as head and mesosoma combined) and the extremely long last tergite (4.4–5.8× basal width) of the gaster.

Type material.—Holotype ♀, Weihe, Heilongjiang Province, Yang Zhong-qi and Wang Xiao-yi , 22 April 2004, ex: Its mature larva collected in the gallery of *A. planipennis* (Note: It was reared from a field collected overwintering mature larva found adjacent to a mummified larvae of *A. planipennis* which had attacked and formed a gallery in the trunk of *Fraxinus mandshurica* on 22 March 2004, and the adult wasp emerged 22 April 2004). Paratypes: 3 ♀, data is the same as holotype; 5 ♀, 1 ♂, Jingyuetan, Jilin Province, larvae collected 19 March 2004, adults emerged 23 April 2004, other data as holotype; 8 ♀, 31 ♂, Benxi, Liaoning Province, larvae collected 18 March 2004, adults emerged 17–19 April 2004, other data as holotype; following all collected in Jingyuetan Forest Park, Changchun, Jilin Province by Yang Zhong-qi and Wang Xiao-yi, 6 ♂, pupae collected from single EAB gallery 18 Sep. 2004, adults emerged 27 Sep. 2004; 8 ♀, 2 ♂, larvae collected from single EAB gallery 18 Sep. 2004, pupated 29 Sep. 2004, adults emerged 13 Oct. 2004; 20 ♀, 10 ♂, larvae collected from single EAB gallery 18 Sep. 2004, pupated 30 Sep. 2004, adults emerged 15 Oct. 15; 32 ♀, 13 ♂, larvae collected from single EAB gallery 18 Sep. 2004,

pupated 29 Sep. 2004, adults emerged 15 Oct. 2004; 66 ♀, 26 ♂, reared from a single EAB mature larva, 12 Oct. 2004 (Note: the EAB larva collected in its gallery on tree of *F. manschurica* 18 Sep. 2004, the parasitoid larvae inside the EAB larva broke through the remaining translucent EAB larva body skin 25 Sep. 2004, pupated 28 Sep. 2004, adults emerged 12 Oct. 2004); 48 ♀, 30 ♂, same date as the previous series except parasitoid larvae broke through the EAB larva 28 Sep. 2004, pupated 5 Oct. 2004, adults emerged 22 Oct. 2004.

The holotype and most paratypes of the new species are deposited in the Insect Museum of the Chinese Academy of Forestry, Beijing, China, with the exception the following paratypes: 6 ♀, 3 ♂, National Museum of Natural History, Smithsonian Institution,Washington, DC, USA; 6 ♀, 3 ♂ Canadian National Collection of Insects (CNCI), Agriculture and Agri-Food Canada, Ottawa, Canada; and 1 ♀, 1 ♂ West Virginia University Arthropod Collection, Morgantown, WV, USA.

Biological observations.—*Tetrastichus planipennisi* is a gregarious endoparasitoid of *Agrilus planipennis* larvae, producing 56–92 individuals from a single host. Feeding by the parasitoid larvae inside the EAB larva did not kill their host until it became a late mature larval stage (4th instar), eventually completely consuming the host body contents until only the host larval translucent integument remained, in which the mature maggot-like larvae (3–4 mm) are clearly visible (Fig. 26). Wasp larvae then exited their host and soon pupated adjacent to its intact membranous integument, which is typically near the end of the gallery. It took approximately 15 days for adult wasps to eclose. The newly emerged wasp chewed a hole through the bark to exit the host gallery, with other emerging wasps in the brood often using the same hole. We estimate that at least four generations are produced a year in northeastern China, with the last generation overwintering as mature larvae inside their host's gallery. The sex ratio of the emerging adults was skewed towards females 2.5:1. The longevity at 25°C of the adult parasitoids was about 15 days for females and 13 days for males.

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